DNA Database Procedure for Autosomal DNA STR Interpretation with PowerPlex® Fusion

1.0 Purpose - To provide guidelines for the interpretation of autosomal DNA results within the DNA Database.

2.0 Scope - This document applies to qualified DNA Database Section Forensic Scientists and trainees.

3.0 Definitions

- **Allele:** An alternative form of a gene; allele designation is used to designate a specific size fragment of DNA for a specific locus in STR analysis.
- **Allelic Dropout:** An occurrence where one or more alleles from an individual's DNA profile fail(s) to amplify during PCR and as a result(s) is(are) not detected in the profile.
- **Amelogenin:** Gender-determining locus.
- **Analytical Threshold (AT):** The minimum height (RFU) requirement at and above which detected peaks may be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles. The threshold for this Laboratory is internally derived by empirical data.
- **Artifact:** Non-allelic byproducts of PCR technology (e.g., stutter), anomalies which occur during capillary electrophoresis (e.g., pull-up, spike), or byproducts of primer synthesis (e.g., dye blob).
- **Core Loci:** The 13 loci defined by the FBI and required for inclusion within CODIS. The 13 core loci are CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11.
- **DNA Profile:** The combination of genotypes obtained from DNA analysis testing of multiple loci.
- **Dropout Threshold (DT):** The peak height value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele of a heterozygotic pair has not occurred. The threshold for this Laboratory is internally derived through the use of empirical data.
- **Full Profile:** A DNA profile that exhibits genotypic information at each locus tested and there is no evidence of allelic dropout, degradation, or preferential amplification.
- **Incomplete Spectral Separation/Pull-up:** A signal from an allele labeled with one dye-set observed as a peak or Off-Ladder Allele in another dye-set.
- **Inhibition:** The total or partial suppression of the PCR process that would result in partial or no DNA profile being obtained.
- **Injection:** When a DNA sample is electrokinetically introduced into a capillary for electrophoretic separation.
- **Locus (plural=Loci):** The chromosomal location or location of a gene or DNA marker.
- **Match:** DNA profiles are considered to match if their patterns are the same after taking into consideration the properties of the substrate tested and limitations of the specific techniques used.
- **Microvariant:** An allele that varies by less than the consensus repeat unit.
- **Mixture:** A DNA typing result originating from more than one individual. **NOTE**: If a DNA profile is observed to have more than two peaks at more than one locus, then there is a high possibility that there is a mixture of two or more individuals' DNA profiles. If three peaks are observed at only one locus, then there may not be a mixture; the individual contributor may have a tri-allelic pattern at that locus.
- **Noise:** Background signal detected by a data collection instrument.
- **Non-Match:** Assuming a single source from a sample, two DNA profiles are considered to be a non-match if there is a difference of one allele after taking into consideration the circumstances of collection and preparation of samples and knowledge of the properties of the substrate tested and limitations of the specific techniques used.
- **Off-Ladder Allele** - Alleles that size within a locus marker size range but outside the allele categories (bins) represented in the ladder.

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- **Off-Scale Data:** The result of excess DNA present in an electrophoresed sample, typically visualized by excessive artifacts as a result of peak heights consistently greater than 12,000 RFUs.
- **Outside Marker Range (OMR)** – Indicates if labeled peaks are detected between two marker size ranges defined in the panel, below the lowest marker size range defined in the panel, or above the highest marker size range defined in the panel.
- **Partial DNA Profile:** A DNA profile that does not produce DNA typing results for all loci tested.
- **Peak:** A well-defined point on an electropherogram that is within bin (on-ladder). See "Microvariant" and "Off-Ladder Alleles" for exceptions to the "on-ladder" requirement.
- **Run:** Each set of 24 samples that are injected and separated electrophoretically on the Capillary Electrophoresis Unit (ABI 3500xL or equivalent).
- **Shoulder and Tail:** Elongated or raised areas to the immediate left and right, respectively, of a main peak, but not separated from the main peak.
- **Spike/Electrical Spike:** An artifact believed to be caused by a spike in the current within a capillary that causes a sharp increase in signal. This artifact lacks the defined morphology of a peak.
- **Split peaks:** A split peak occurs when one allele is represented by two peaks. Lack of full "nucleotide A" addition may be observed when the amount of input DNA is greater than the recommended protocol. In this case, more time is needed for Taq Polymerase to add the "A" nucleotide to all molecules. Amplification of too much input DNA also results in off-scale data (saturation of signal) and may be manifested as split peaks.
- **Single Source Profile:** A combination of genotypes obtained from STR DNA testing that could only originate from a single individual. A sample may be considered to consist of a single contributor when no more than two alleles are observed at each locus. All loci are to be evaluated in making this decision.
  If three alleles are observed at one locus, then there may not be a mixture; the individual contributor may have a tri-allelic pattern at that locus.
- **Stochastic Effects:** The observation of intra-locus peak imbalance and/or allele drop-out resulting from random, disproportionate amplification of alleles in low-quantity template samples.
- **Stutter:** An artifact of PCR amplification that is typically one repeat unit less than the corresponding main allele peak resulting from strand slippage during amplification.
- **Tri-allelic Pattern:** Three peaks observed at a single locus and not the result of a mixture. These peaks may or may not be of equal intensity.
- **Unincorporated Dye:** Unincorporated dye (i.e., dye-blobs) may be observed in an electropherogram and are distinct morphologically from a labeled DNA fragment. A dye-blob does not exhibit the typical sharp, distinct peak that is produced by actual alleles and is observed as a wider, thicker peak and may be lacking the sharply defined slope to the apex of a peak.
- **Y-Specific Marker:** A locus on the Y chromosome. Only samples containing male DNA will produce alleles for this type of marker.

4.0 Equipment, Materials and Reagents - N/A

5.0 Procedure

5.1 Introduction

The guidelines outlined herein are based upon this Laboratory's validation studies, review of literature, and over 20 years of forensic DNA Database experience. These guidelines are to be used in conjunction with the DNA Database Forensic Scientist's training and experience to provide scientific interpretation of the STR results.

5.2 3500xL Thresholds

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5.2.1 Analytical Threshold

The analytical thresholds were established through validation and performance check studies using the PowerPlex® Fusion PCR Amplification kit.

5.2.1.1 The analytical threshold for the orange dye channel (WEN ILS 500) shall be set at 100 RFU.

5.2.1.2 The analytical threshold for 18s injections is set at 125 RFU for blue, green, yellow, and red dye channels. Anything present below 125 RFU is considered to be indistinguishable from background noise and shall not be considered for analysis.

5.2.2 Dropout Threshold

The dropout threshold for 18s injections is set at 180 RFU.

NOTE: This threshold does not apply to the locus DYS391.

5.3 Interpretation of Samples, Controls, and Allelic Ladders

5.3.1 Examination of the Electropherogram of the Allelic Ladder(s)

All alleles within the allelic ladder for all loci tested shall be 1) equal to or greater than the analytical threshold and 2) in the correct position in order to use the associated samples and controls. Allelic ladders shall be analyzed as specified in the DNA Database Procedure for GeneMapper ID-X.

5.3.2 Examination of the Electropherogram of the Positive Amplification Control

All positive amplification controls shall be void of extraneous, detectable alleles. If multiple positive amplification controls are run, at least one must yield a complete profile. If no positive amplification controls produce a complete profile, data from the associated runs shall not be used.

5.3.3 Examination of the Electropherogram of the Negative Controls

If any peaks not attributable to artifacts are above the analytical threshold in the amplification negative control or the reagent blank samples, the controls and sample(s) shall be reanalyzed (i.e., reinjected or reamplified). If it is not possible to reanalyze the data because of sample depletion, the DNA Database Forensic Scientist may proceed to interpret the results of the samples upon consultation with the Technical Leader.

Artifacts observed in the negative controls do not require the samples to be reinjected. Those artifacts shall be documented in the notes.

5.3.4 Examination of the Electropherograms of Samples

Assess the quality of the peaks including RFU values and determine if artifacts are present. (Refer to the DNA Database Procedure for GeneMapper ID-X.)

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Single alleles which fall below the dropout threshold shall be considered partial for purposes of interpretation and CODIS entry. In such instances loci shall be clearly marked (highlighted) on the allele call table. Single alleles which are above the dropout threshold shall be considered true homozygotes and not potential candidates for allelic dropout.

For Database samples, the profile shall contain the 13 core loci for upload into CODIS.

The use of off-scale Database samples shall be at the discretion of the DNA Database Forensic Scientist based upon training and experience.

Triallelic samples shall be reamplified as a second, confirmatory analysis if there is no previous data for the sample/locus or the previous data does not display a triallele at that locus.

It is permissible to combine results from different injections and amplifications of the same sample when determining a final DNA profile.

5.4 Artifacts

The PCR process produces artifacts that are known and well-characterized. All by-products of PCR and/or capillary electrophoresis shall be labeled on electropherograms as "artifact."

5.4.1 Stutter – The Genemapper ID-X software contains designated cutoffs for peaks in stutter positions and shall be used for designating stutter. Stutter products are most often observed one repeat unit below the true allele peak. Note that D22S1045 is a trinucleotide and that Penta D and Penta E are pentanucleotides.

5.4.1.1 N+4 peaks – For tetranucleotides, a stutter peak may appear in the n+4 position.

5.4.1.2 N+3 peaks – For trinucleotides, a stutter peak may appear in the n+3 position.

5.4.2 Known Artifacts – Promega has noted reproducible artifacts that may appear in samples amplified with PowerPlex® Fusion. These artifacts may be observed in the locations specified below.

5.4.2.1 N±2 peaks - D1S1656, D13S317, D18S51, D21S11, D7S820, D5S818, D12S391 and D19S433

5.4.2.2 N-1 peaks – Amelogenin and D2S441

5.4.2.3 N-3 peaks – D12S391

5.4.2.4 For a list of additional artifacts that are typically below common minimum thresholds, see the PowerPlex® Fusion System Technical Manual.

5.4.3 Incomplete Spectral Separation/Pull-up - Generally, pull-up may be noted when all the alleles are overlapped using the software and the pull-up is observed as a relatively small peak located directly under the larger peak. Scientists shall be knowledgeable of this phenomenon and use the computer software to aid in discerning actual alleles from pull-up.

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5.4.4 Unincorporated Dye - Scientists shall not call dye-blobs as an actual allele. Dye-blobs shall not be considered for interpretation.

5.4.5 Shoulder and Tail - Shoulders and tails do not prevent the scientist from assigning the specific peak an allelic value.

5.5 Heterozygote Peak Balance – Database samples generally exhibit a balance of >70 % between heterozygous alleles. A database sample shall be rerun if the imbalance does not allow for proper interpretation by the scientist.

6.0 Limitations – N/A

7.0 Safety – N/A

8.0 References

Butler, J.M. *Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers*. 2nd ed. Burlington, MA: Elsevier Academic Press, 2005.

DNA Database Section Procedure for DNA Database Operations

DNA Database Section Procedure for GeneMapper ID-X

Federal Bureau of Investigation. "QUALITY ASSURANCE KNOWN SAMPLES FOR DNA DATABASING LABORATORIES." *September 1, 2011*.

Procedure for CODIS

PowerPlex Fusion System: Instructions for Use of Product DC2402 and DC2408. 2012 Promega Corporation. Part Number TMD039 Rev.10/12. (or most recent revision)

9.0 Records – N/A

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
08/03/2015	1	Original Document
04/18/2016	2	5.2.1 and 5.2.2 removal of 24 sec injection; updated CC5 to WEN for size standard

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